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14. ABSTRACT Acute alcohol intoxication (AAI) impairs the hemodynamic countereulatory response to trauma and hemorrhagic shock (HS), blunts the pressor response to fluid resuscitation (FR), suppresses the HS-induced neuroendocrine response, impairs pro-inflammatory cytokine expression and increases mortality from infection during recovery. Studies conducted during this funding period examined a) whether the attenuated neuroendocrine response, particularly reduced sympathetic nervous system (SNS) activation, is the principal mechanism responsible for the hemodynamic instability seen in AAI+ HS and b) what the impact of AAI was on the integrity of host defense mechanisms during the immediate and delayed recovery from HS. We determined whether SNS activation can be restored by central (intracerebroventricular; ICV) neostigmine administration and whether this in turn is capable of improving the hemodynamic countereulatory response to HS in AAI. Our results show that ICV neostigmine stimulates SNS activation and improves the recovery of blood pressure following hemorrhagic shock. Furthermore, our results indicate that this in part mediated by arginine vasopressin. Interestingly while the presser response to phenylephrine in vitro appears to be blunted by alcohol, the in vivo response to a presser with a different mechanism of action appears to be preserved.					
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INTRODUCTION: Acute alcohol intoxication (AAI) impairs the hemodynamic counterregulatory response to trauma and hemorrhagic shock (HS), blunts the pressor response to fluid resuscitation (FR), suppresses the HS-induced neuroendocrine response, impairs pro-inflammatory cytokine expression and increases mortality from infection during recovery. Studies conducted during this funding period examined a) whether the attenuated neuroendocrine response, particularly reduced sympathetic nervous system (SNS) activation, is the principal mechanism responsible for the hemodynamic instability seen in AAI+ HS and b) what the impact of AAI was on the integrity of host defense mechanisms during the immediate and delayed recovery from HS. We determined whether SNS activation can be restored by central (intracerebroventricular; ICV) neostigmine administration and whether this in turn is capable of improving the hemodynamic counterregulatory response to HS in AAI. Our results show that ICV neostigmine stimulates SNS activation and improves the recovery of blood pressure following hemorrhagic shock. Furthermore, our results indicate that this is in part mediated by arginine vasopressin. Interestingly while the pressor response to phenylephrine in vitro appears to be blunted by alcohol, the in vivo response to a pressor with a different mechanism of action appears to be preserved.

Progress report 2nd funding period:

The PI would like to highlight that our laboratory is now fully operational following Hurricane Katrina. Similar to last year, we have performed studies that fall under the 3 objectives of the proposal. Progress made in these will be described accordingly.

Objective 1: To test the hypothesis that acute alcohol intoxication alters central activation of descending sympathetic outflow. The proposed studies will identify the mechanisms responsible for the impaired hemodynamic counterregulatory response to blood loss in the alcohol-intoxicated host. Specifically, to isolate central and peripheral regulatory mechanisms disrupted during alcohol intoxication.

- a. Determine whether direct central activation of sympathetic outflow restores catecholaminergic and hemodynamic responses to hemorrhagic shock in alcohol-intoxicated animals.
- b. Determine whether inhibition of central sympathetic activation during alcohol intoxication is mediated through enhanced tonic inhibition by nitric oxide.
- c. Examine whether central administration of arginine vasopressin enhances sympathetic activation and restores catecholaminergic and hemodynamic responses to hemorrhagic shock in alcohol-intoxicated animals.

Progress:

Research Objective 1a and 1c.

Central neostigmine administration reverses alcohol- and hemorrhage-induced hypotension. One of the most critical determinants of outcome within the first 48 hours of injury is the victim's mean arterial blood pressure (MABP) at the time of admittance into the emergency department. Previously we have demonstrated that ICV choline increased basal MABP (+17%) and produced a similar increase in basal MABP in alcohol intoxicated. However, ICV choline did not alter the initial % decrease in blood pressure nor did it improve MABP throughout hemorrhagic shock or fluid resuscitation in alcohol-treated animals.

These studies showed that intracerebroventricular (ICV) choline (acetylcholine precursor) administration produced a transient activation of sympathetic nervous system outflow insufficient to improve MABP following AAI + HEM. We determined whether enhancing acetylcholine availability by ICV neostigmine (acetylcholinesterase inhibitor) would improve MABP following AAI and HEM. The dose response to ICV neostigmine (0.1-3 µg) was established in chronically-catheterized, conscious male Sprague-Dawley rats

(225-250g) and 1 μg was selected based on its ability to produce an immediate (10 min) and sustained (2 h) increase (20%; $P=0.07$) in MABP. As shown in figure 1, ICV neostigmine reversed the 12% ($P=0.001$) drop in MABP caused by alcohol (2.5 g/kg, 30% v/v) administration within 30 min, completely reversed the hypotension produced by 40% total blood loss, and improved 7-day survival (100% vs. 25%) from HEM. These results demonstrate that ICV neostigmine reverses hemorrhage- and alcohol-induced hypotension and produces a more sustained elevation in MABP.

Presser responses produced by central cholinergic activation are in part mediated by arginine vasopressin.

Preliminary follow up studies suggest that the effects of neostigmine on blood pressure may be mediated in part by arginine vasopressin. In a different set of animals, administration of a selective AVP receptor antagonist (V_{1a} ; 10 $\mu\text{g/kg}$) blunted the neostigmine (1 μg) induced rise in BP. Those findings indicated that AVP contributes to the pressor response elicited by central neostigmine administration. Whether this is preserved in alcohol-intoxicated animals is currently under investigation. Furthermore, neostigmine produced a marked elevation in Epi ($315\pm 88\%$; $P=0.001$) and NE ($89\pm 29\%$, $P=0.005$) levels, suggesting that its pressor response cannot be solely attributed to enhanced AVP release.

Objective 2: Examine the impact of alcohol intoxication on vascular responsiveness to pressor agent administration.

- To determine the impact of acute alcohol intoxication during trauma/hemorrhage on vascular responsiveness to in vivo administration of pressor agents (norepinephrine and arginine vasopressin).
- To examine the impact of alcohol on vascular reactivity to direct application of pressor agents to isolated vessels.

Progress: objective 2a

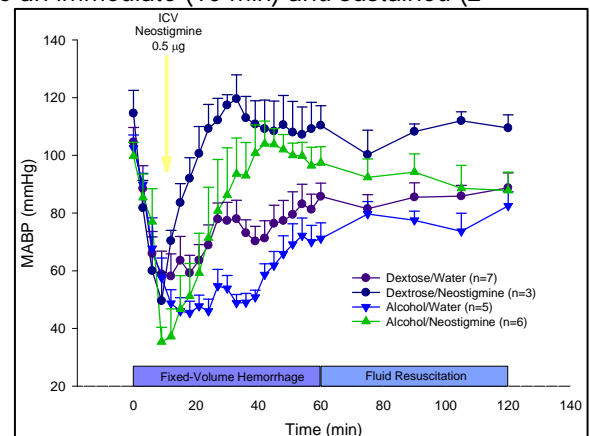


Figure 1. Central cholinergic activation increases MABP following fixed volume hemorrhage in control and alcohol-intoxicated animals.

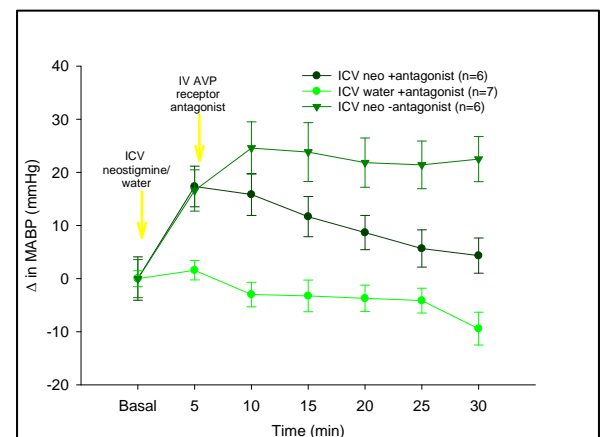


Figure 2. Administration of an antagonist to the arginine vasopressin receptor blunts the rise in blood pressure resulting from ICV neostigmine administration.

Systemic administration of arginine vasopressin improves pressor response following hemorrhage.

The impaired hemodynamic counterregulation to HEM in AAI rodents is associated with attenuation of circulating levels of arginine vasopressin (AVP), epinephrine (Epi) and norepinephrine (NE). We hypothesize that restoration of the neuroendocrine response, either through central activation or systemically through vasopressor administration, will improve BP recovery and outcome from HEM during AAI. Studies were conducted to determine the efficacy of AVP in restoring the pressor response following hemorrhage. A constant intravenous infusion of AVP (0.01 U/kg/min) was administered to catheterized, conscious male Sprague-Dawley rats following a fixed-pressure (40 mmHg) HEM. As shown in figure 3, both experimental groups responded with similar increases in blood pressure. Furthermore, it appears that

the alcohol-intoxicated animals had higher blood pressure levels beyond completion of resuscitation. This would suggest that responsiveness to AVP is not compromised in alcohol-intoxicated animals.

Progress objective 2b

Alcohol affects vasoconstrictive and vasodilatory responses in isolated aortic and mesenteric rings. This study examined the effects of AAI on blood vessel reactivity to phenylephrine (PE), acetylcholine (Ach), and nitroprusside (NP) ex-vivo. Chronically instrumented, conscious male Sprague-Dawley rats (300-350 g) received a primed continuous 15 hour intragastric ALC infusion (1.75g/kg + 300 mg/kg/hr). Time-matched controls received isocaloric/isovolumic dextrose. Thirty minutes after discontinuing ALC, animals were sacrificed for isolation of thoracic aorta and mesenteric arteries. Aortic and mesenteric ring segments (1-2 mm) were suspended in myograph baths containing Krebs-Henseleit bicarbonate buffer, pH 7.4, gassed with 95% O₂: 5% CO₂. Arterial rings from AAI rats had decreased PE-induced tension (aorta: 2.28 ± 0.09 vs. 2.6 ± 0.13 g; mesenteric artery: 1.70 ± 0.06 vs. 1.91 ± 0.10), greater (21%) Ach-mediated relaxation, and similar NP-mediated relaxation. These results indicate that AAI favors vasodilatation and are consistent with enhanced endothelial dilator function.

Current studies are examining the vasoconstrictive and vasodilatory responses in isolated aortic and mesenteric arteries following hemorrhage and fluid resuscitation in control and alcohol-intoxicated animals.

Research Objective 3: To test the hypothesis that the alterations in hemodynamics produced by acute alcohol intoxication during trauma-hemorrhage result in inadequate tissue perfusion during the resuscitation period leading to enhanced susceptibility to tissue injury.

- a. Examine the impact of acute alcohol intoxication on tissue blood flow redistribution following fluid resuscitation.
- b. Identify the host defense mechanisms affected by alcohol intoxication & traumatic injury that impair the ability to effectively respond to a "second hit" infectious challenge.

Progress Objective 3b.

Previously, we demonstrated that acute alcohol intoxication prior to hemorrhagic shock impairs hemodynamic and neuroendocrine counterregulation, suppresses early lung pro-inflammatory cytokine expression and increases mortality from infection during recovery. Alcohol-binge prior to hemorrhagic shock (HS) has been shown to suppress early pro-inflammatory cytokine expression and increase mortality

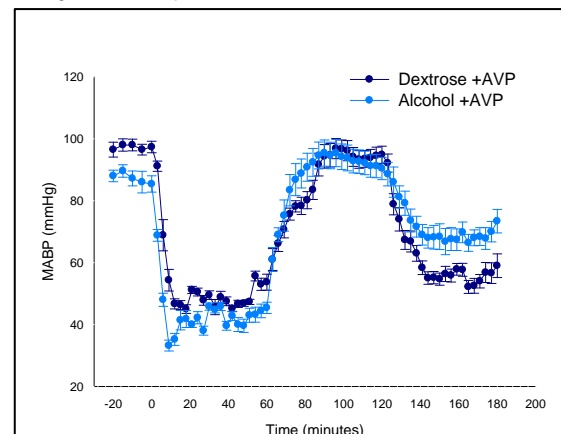


Figure 3. Intravenous administration of AVP improves recovery of blood pressure following hemorrhage in both control and alcohol-treated animals.

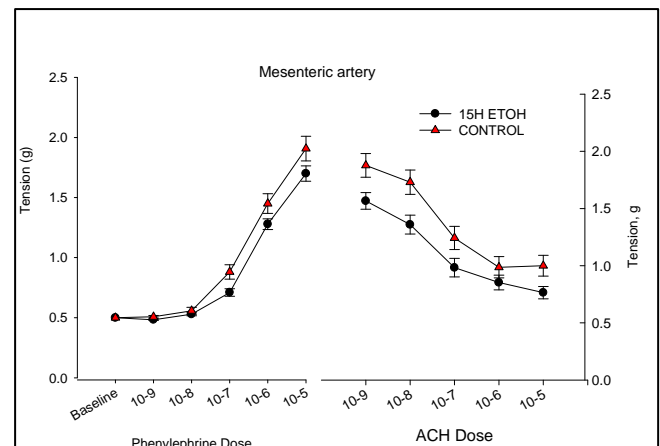


Figure 4. Dose-dependent increases in peak tension and peak relaxation in response to phenylephrine and acetylcholine in mesenteric artery rings obtained from non-hemorrhaged animals.

from infection during recovery. Recently we have observed suppression of peripheral blood mononuclear cells' (PBMC) pro-inflammatory cytokine response to a "second-hit" inflammatory challenge 1 day after Trauma-HS (TxHS). This study examined the effects of alcohol-binge prior to TxHS on the immune responsiveness of PBMCs 5 days after injury. Chronically-catheterized adult, male Sprague-Dawley rats were subjected to an alcohol-binge (5g/kg intragastric 30% w/v for 3 consecutive days, 2.5g/kg on day 4, 30 min prior to TxHS). Time-matched controls received isocaloric/isovolumic dextrose (Dex). PBMCs from animals following a 5 day recovery period were incubated with and without purified LPS (1ug/mL) in 24-well plates and cytokine concentrations were measured in culture supernatants at 6 and 24 h. Significant LPS-stimulated release of TNF, IL-1, and IL-6 was observed in cells obtained from all experimental groups. No differences in concentrations of TNF, IL-1, or IL-6 were noted between supernatants of cultured PMCs isolated from sham and TxHS animals. In contrast, alcohol-binge animals had increased TNF (90%; $p<0.05$), IL-1 (53%; NS), and IL-6 (38%; NS) supernatant concentrations when compared to their dextrose-treated counterparts. Similarly, alcohol-binge+TxHS animals had increased TNF (31%; NS), IL-1 (56%; $p<0.05$), and IL-6 (72%; $p<0.05$) supernatant concentrations when compared to their Dex-treated/TxHS counterparts. Taken together, the results from this and our most recent studies show early suppression and delayed upregulation of LPS-stimulated PBMC cytokine responses following alcohol-binge. These findings reflect apparently contrasting time-related immune-modulating effects of alcohol-binge. Furthermore, the effects of alcohol-binge appear to synergize with TxHS, as seen in the sustained derangement of host inflammatory response following relatively modest injury. We speculate that these immunomodulatory effects will compromise recovery from a combined alcohol-binge+TxHS by increasing host susceptibility to a "second-hit" well into the post-injury recovery period.

Key research accomplishments:

- Demonstrated that neostigmine reverses hypotension produced by blood loss in control and alcohol-intoxicated animals
- Demonstrated that the effects of neostigmine are mediated in part by arginine vasopressin receptor activation
- Demonstrated that neostigmine produces increases in catecholamines, but these are not equally accentuated following hemorrhage
- Demonstrated that survival from hemorrhage in alcohol-intoxicated animals can be significantly improved by restoring blood pressure closer to basal values
- Demonstrated that alcohol decreases the peak tension achieved with vasopressors and accentuates the vasodilation observed with acetylcholine
- Demonstrated that in vivo, the responsiveness to arginine vasopressin appears to be preserved in alcohol-intoxicated hemorrhaged animals.

Reportable outcomes

Publications:

Greiffenstein P, Mathis KW, Vande Stouwe C, Molina PE. Alcohol binge prior to trauma/hemorrhage impairs integrity of host defense mechanisms during recovery. *Alcoholism: Clinical and Experimental Research*. 31(4): 1-12, 2007.

Greiffenstein P, Molina PE. Alcohol-Induced Alterations of Host Defense Following Traumatic Injury. *The Journal of Trauma, Injury, Infection and Critical Care* (in press)

Presentations:

KW Mathis, P Greiffenstein, C Vande Stouwe, PE Molina. Noradrenergic modulation of systemic response to infection. Society on Neuroimmune Pharmacology. Salt Lake City, April 2007.

KW Mathis and PE Molina. Short-term central activation of descending sympathetic outflow does not restore alcohol-induced hemodynamic instability during hemorrhagic shock. FASEB, Washington, DC, April 2007.

KW Mathis and PE Molina. Acute alcohol intoxication impairs counterregulatory responses during hemorrhagic shock. Graduate Research Day, LSU Health Sciences Center, New Orleans, LA, April 2007.

Molina PE. Consequences of alcohol intoxication during trauma/hemorrhage. Shock Society Meeting Satellite, Baltimore, MD, June 2007.

K Mathis and P Molina. Transient sympathetic nervous system activation does not alter early tissue inflammatory responses to hemorrhagic shock. Shock Society Meeting, Baltimore, MD, June 2007.

KW Mathis and PE Molina. Central choline-mediated sympathetic outflow does not attenuate the hypotensive response to blood loss in acute alcohol-intoxicated rodents. Alcohol and Trauma 2007, Shock Society Satellite Meeting, Baltimore, MD, June 2007.

KW Mathis and PE Molina. Central choline-mediated sympathetic outflow does not attenuate hypotensive response to blood loss in acute alcohol-intoxicated rodents. Research Society on Alcoholism. Chicago, IL, July 2007.

KW Mathis and PE Molina. Central manipulation of the sympathetic nervous system during hemorrhagic shock in the alcohol-intoxicated host. NIAAA Trainee Workshop, Indianapolis, IN, September 2007.

Molina PE. : Alcohol intoxication and traumatic injury; Hemodynamic, metabolic and immune dysregulation. European Society for Biomedical Research in Alcoholism, Berlin, Germany, September 2007.

Molina PE. Impact of alcohol intoxication on outcome from trauma/hemorrhage. Tulane University, New Orleans, February 2008.

Conclusions:

The results from our ongoing studies have provided evidence that central cholinergic stimulation may be an effective intervention to enhance sympathetic outflow in alcohol-intoxicated hemorrhaged animals. In addition, the results suggest that both central and systemic mechanisms may be involved in impaired counterregulatory responses in alcohol-intoxicated hemorrhaged animals. Our most recent findings from those studies have shown that central administration of the acetylcholinesterase inhibitor, neostigmine; which produces central cholinergic activation restores blood pressure and sympathetic nervous system (SNS) activation during hemorrhage. However, because central pharmacological interventions are not feasible or practical in the clinical setting, studies to be conducted in the next funding cycle will circumvent that by using systemic administration of cholinergic agonists (physostigmine) that cross the blood-brain barrier in conjunction with systemic cholinergic antagonists to prevent untoward effects of enhanced acetylcholine availability in peripheral tissues. The proposed translational studies, are based on data generated from studies funded by this DoD grant, but will further characterize the efficacy of this intervention in restoring pressor responses, tissue perfusion, and survival following shock. Furthermore, they will investigate whether the improvement in blood pressure recovery during fluid resuscitation is dependent on time of pharmacological intervention and whether it results in improved tissue perfusion. The results generated will provide important pre-clinical data with potential for therapeutic development.